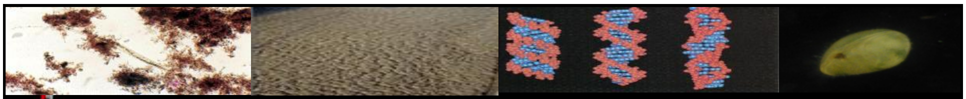
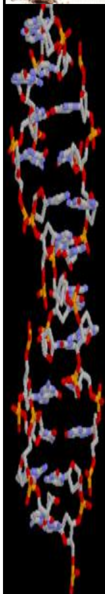
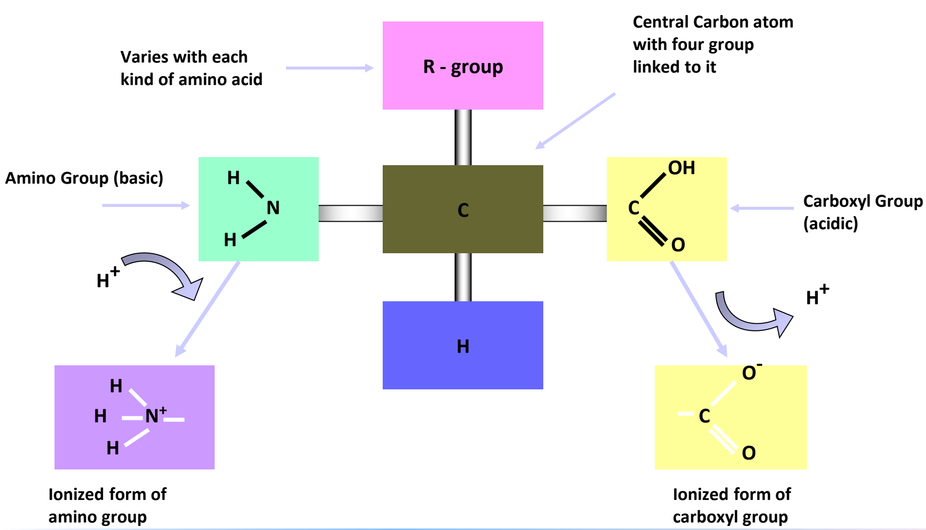




CE665A
ECOLOGICAL AND BIOLOGICAL PRINCIPLES AND PROCESSES

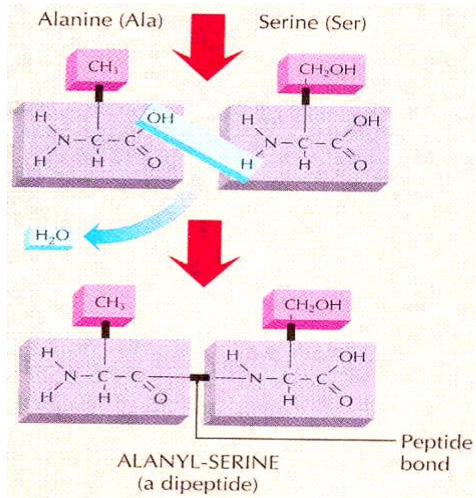
Instructor : Dr Vinod Tare

Proteins → Amino Acids & Peptide Bond



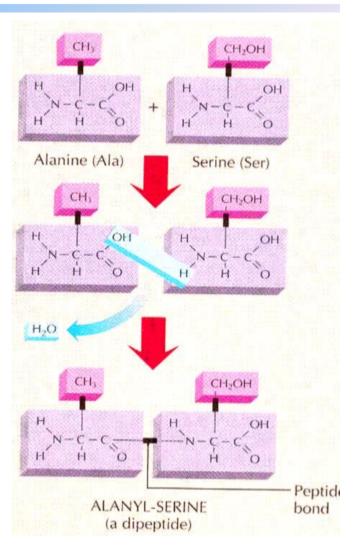
Peptide Bond

- Formed by removal of water molecule, joining amino group of one amino acid to carboxylic group of another amino acid.
- Tie together amino acids to form a long chain, called a polypeptide chain.
- Proteins consist of one or more of these polypeptide chains, which may change in length from fewer than 100 amino acids to more than 1000.



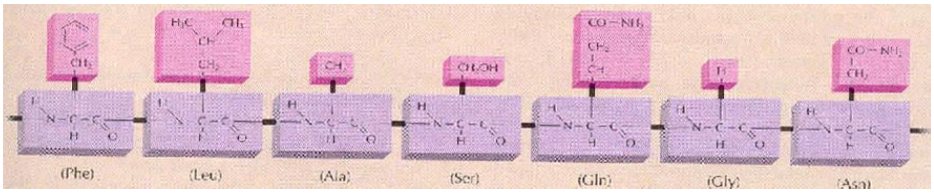
Di-peptide

By removing a molecule of water, two amino acids can be linked together to form a dipeptide.



Polypeptide

Many amino acids can be linked together to form a long chain, called a polypeptide.



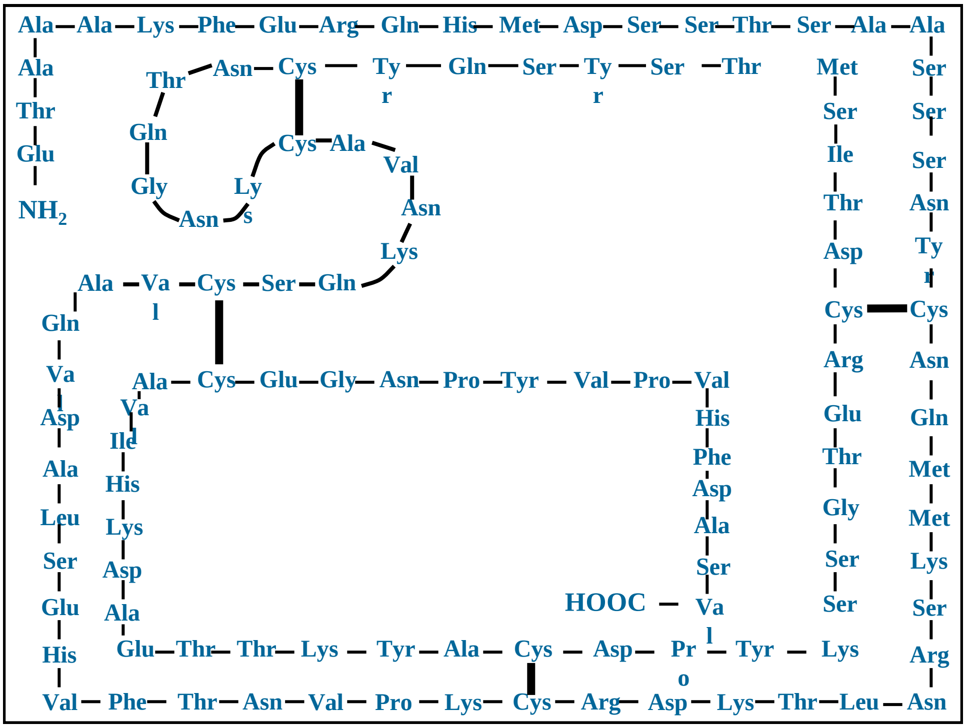
(Phe) (Leu) (Ala) (Ser) (Gln) (Gly) (Asn)

Portion of polypeptide chain

Levels of Protein Structure

A living cell contain 1000 or more different kinds of proteins, and each kind has its own unique sequence of amino acids. The amino acids sequence is called the primary structure of the protein.

(for e.g. ribonuclease contains 124 amino acids in a specific sequence).

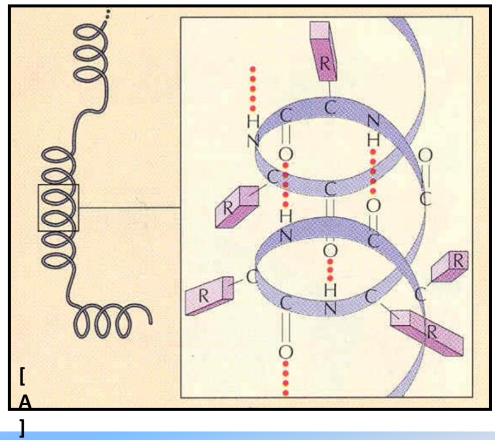


Protein Structure

- **Secondary structure:** A polypeptide chain can fold into a specific shape, much like a ribbon. Some portions of chain may form a coil, while others may form a side-by-side arrangements or other configurations. These forms constitute the secondary structure and are due to H bonding between polar –C=O and –NH groups along the chain.
- **Tertiary structure:** Overall folding of molecules into a specific shape, caused by interaction between different types of polypeptide chains. For instance, di sulfide bridges or bonds between sulphur ions contribute to the tertiary structure by connecting cysteine molecules located in different regions of the polypeptide chain.
- **Quaternary Structure:** Some proteins contain two or more polypeptide chains for their proper activity. This combination of polypeptide chains constitutes the quaternary structure. For example, the blood protein hemoglobin contains four polypeptide chains.

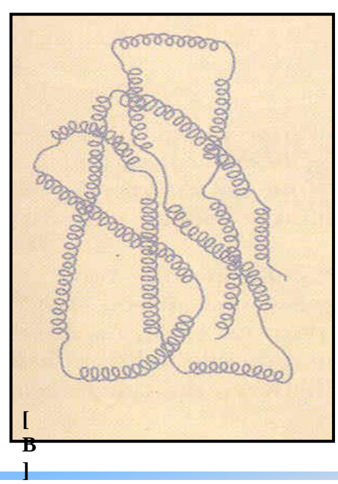
Protein Structure

[A] Secondary structure of a protein. Portions of the polypeptide chain from an alpha helix due to hydrogen bonding (•••••) between –C=O and –NH groups of the peptide bonds. (for simplicity, only the hydrogen atoms actually involved in the hydrogen bonding are shown.) The R Groups of the amino acids in the chain project outward from the helix.



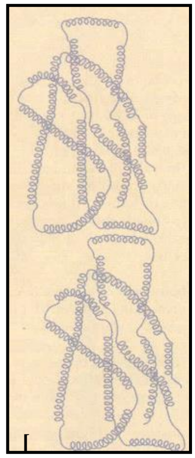
Protein Structure

[B] The tertiary structure of a protein is determined by interactions between portions of the chain



Protein Structure

[C] Quaternary structure of a protein. The protein shown here is composed of two identical polypeptide chains, but some proteins are composed of several different kinds of chains.



[C]

Nucleic Acids

- Discovery of chemical substance that carries genetic information of cells was one of the most exciting findings of the twentieth century (by American microbiologists)
- DNA & another substance, first found in nuclei of cell, ribonucleic acids (RNA), are called nucleic acids.
- DNA (deoxyribonucleic acid) is the substance responsible for the inheritable characteristics of living organisms.
- DNA is the substance that contains the hereditary information of a cell, whereas RNA is usually involved in deciphering the hereditary information in DNA and carrying out its instructions.

DNA

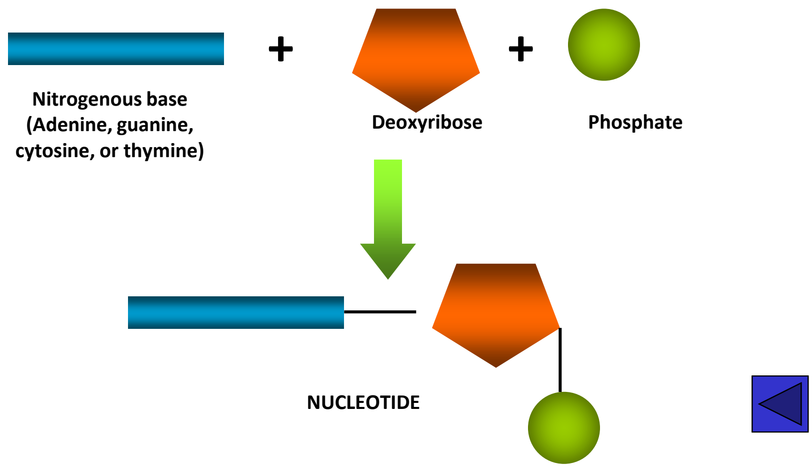
- Longest molecules in living cells (=1000 times longer than the cell itself)
- Fits into the cell because it is twisted into a highly compact form.
- A single molecule of DNA contains a vast library of hereditary information.
- But it has relatively simplest chemical structure.

DNA

Composition:

- A DNA molecule is composed of molecules called nucleotides.
- Each nucleotides consists of three parts
  1. One molecule of a class of nitrogen containing compounds called nitrogenous bases.
  2. One molecule of the pentose sugar deoxyribose.
  3. One phosphate group.
- By using energy from food sources, a cell links these three parts to form a nucleotide.
- Four kinds (two groups) of nitrogen bases occur in DNA
  1. Adenine and Guanine → Called Purines
  2. Cytosine and Thymine → Called Pyrimidines.
- A cell puts together thousands of nucleotides to form a single strand of DNA. Two things are interesting about this strand:
  1. Each phosphate is attached to two deoxyribose. And,
  2. Phosphate and deoxyribose alternate to form a "backbone" from which project the purines and pyrmidines.

Nucleotide

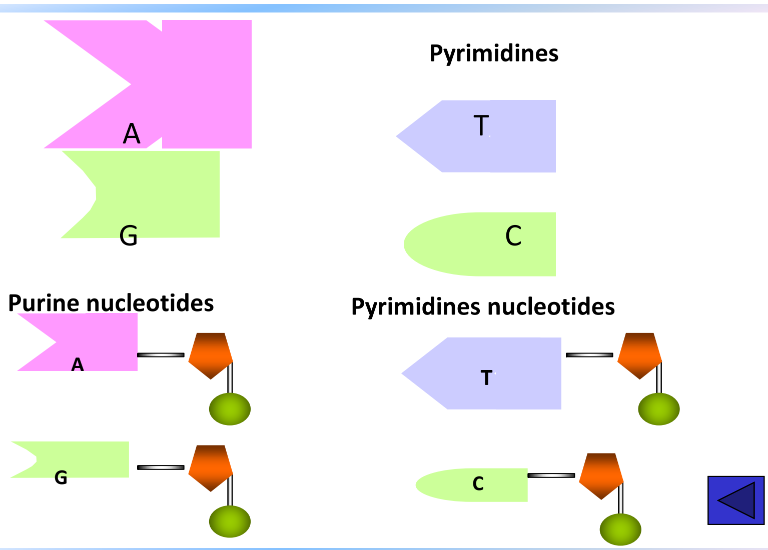


Lecture 8

CE 665A
Ecological and Biological Principles and Processes

Dr Vinod Tare

Nucleotides



Lecture 8

CE 665A
Ecological and Biological Principles and Processes

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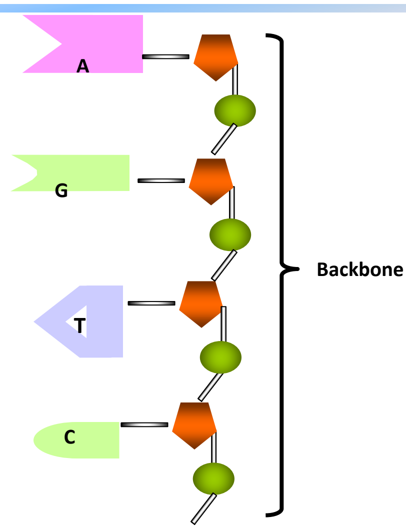
Portion of DNA Strand



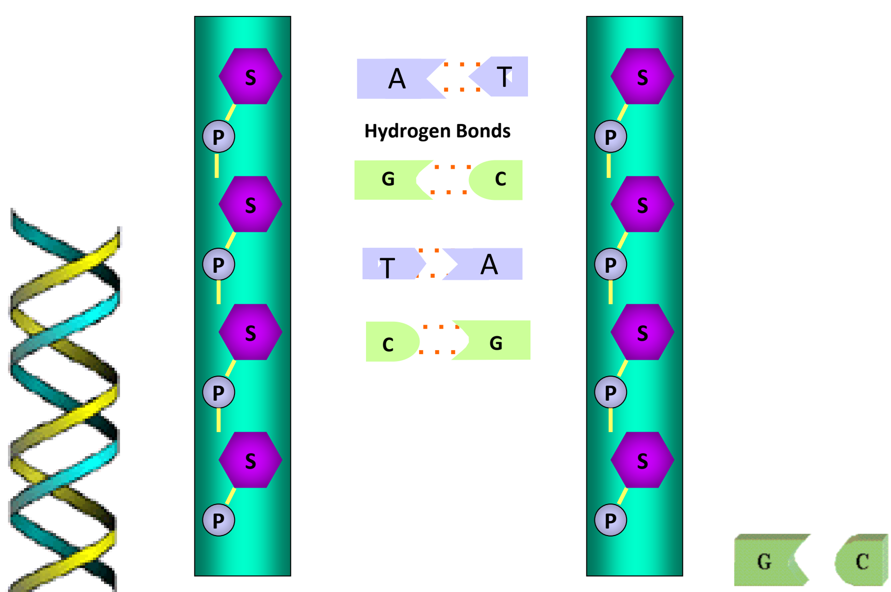
Double Stranded DNA

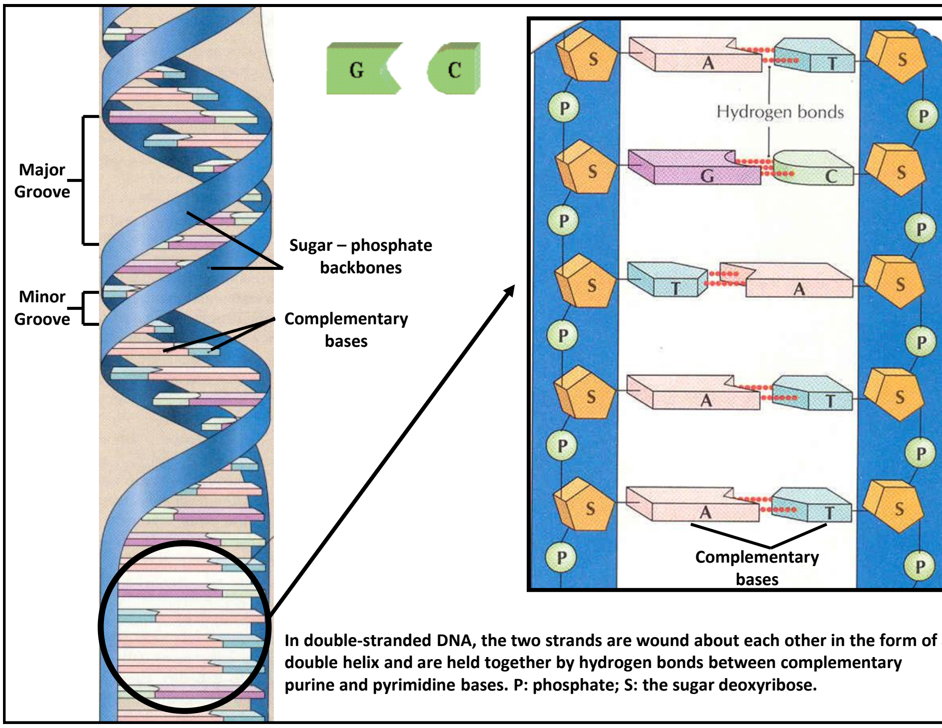
- Finally, two strands are cross-linked by means of the projecting purines and pyrimidne bases to form double stranded DNA.
  - Hydrogen bonds link the bases on one chain with those on the other chain.
  - The two bases attached in this manner are complimentary and called “complimentary base pair”.
  - Only two kinds of complimentary base pairs are found in DNA.
  - Thus ratio of A to T or G to C is always 1:1 in double stranded DNA.
  - The complimentary of the purines and pyrimidines means that the sequence of bases on one strand dictates the sequence on the other.
  - This is of critical importance in the synthesis of new strands of DNA during cell division, because it is the sequence of bases in DNA that represents the hereditary information of cell. There is a different sequence for each species of living organism.

Double Stranded DNA

- In the double-stranded DNA molecule, the two strands are not straight, but are wound around each other to form a double helix (two strands in a double helix are held by H bonds between the complementary bases).

In double-stranded DNA, the two strands were wound about each other in the form of a double helix and are held together by hydrogen bonds between complementry purine and pyrimidine bases. P, Phosphate; S, the sugar deoxyribose





In double-stranded DNA, the two strands are wound about each other in the form of a double helix and are held together by hydrogen bonds between complementary purine and pyrimidine bases. P: phosphate; S: the sugar deoxyribose.

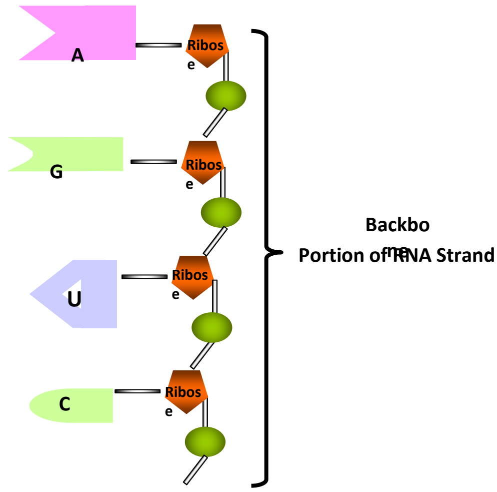
RNA

- RNA is also composed of a chain of nucleotides, but it differs from DNA in certain respects.

1. Sugar in RNA is ribose and not deoxyribose.
2. RNA contains pyrimidine called uracil instead of thymine.
3. Unlike double stranded DNA, RNA is single stranded. This means that there is no complementary second strand paired with it. Thus the ratio of A to U or G to C can vary and is not necessarily 1:1.

RNA

The structure of RNA. RNA differs from DNA by having the sugar ribose instead of deoxyribose, and the pyrimidine **uracil (U)** instead of thymine. The other three bases (A, G, and C occur in both RN and DNA. Unlike DNA, RNA is single standarded.



Enzymes

- Serve as catalytic agents.
- Specific to chemical reactions.
- Some are capable of increasing the rate of a chemical reaction millions of times over that of spontaneous reaction.
- Sensitive to their surroundings.
- Can be inhibited in various ways (Enzyme inhibition) (H_2 & O_2 will make long time to form water, finely divided into powder of Platinum can act as a catalyst to form water instantaneously)
- Unlike inorganic catalyst, enzymes are organic substances produced by living cells.

Enzymes

- Until recently all enzymes were thought to be proteins but recently (in 1989 Sidney Altman of Yale University and Thomas Cech of the university of Colorado received Noble prize in chemistry) it has been discovered that RNA can also catalyze certain reactions in cell. This discovery has revolutionized the ideas held by biochemists about the origin and nature of enzymes.
- Some enzymes are pure proteins, but many consist of a protein combined with a much smaller non-protein molecule (called coenzyme), which assists the protein portion (called the apoenzyme), by accepting or donating atoms when needed.

Apoenzyme + Coenzyme = Holoenzyme

Vitamins

- May be coenzyme or principle components of a particular enzyme.
- Are organic substances that occur naturally in very small amounts but are essential for all cells.
- The vitamins that an organism cannot synthesize, must be supplied in the diet.
- Inorganic coenzymes (Mg, Zn, etc.) are called as cofactors
- Sometimes both a cofactor and a coenzyme are required before an enzyme is able to act as catalyst.

Some Coenzymes and Their Constituent Vitamins

Coenzymes	Vitamin
Coenzyme A (CoA)	Pantothenic acid
Cocarboxylase (thiamine pyrophosphate, TPP)	Thiamine (B_1)
Flavin adenine dinucleotide (FAD)	Riboflavin (B_2)
Nicotinamide adenine dinucleotide (NAD) and nicotiamide adenine dinucleotide phosphate (NADP)	Niacin (nicotinic acid)
Pyridoxal phosphate	Pyridoxal (B_6)
Tetrahydrofolic acid (THF)	Folic acid

Enzymes and Their Classification

- Although there are thousands of kinds of enzymes, they can be grouped into six major classes.
- The name of any enzyme always has the suffix -ase and is usually based on the particular chemical reaction it catalyzes. For example an enzyme that removes hydrogen atoms from Lactic acid are called as ***lactic acid dehydrogenase.***

Major Classes of Enzymes

Class No	Class Name	Catalytic Reaction	Example of Enzyme and the Reaction it Catalyzes
1	Oxidioreductases	Electron-transfer reactions (transfer of electrons or hydrogen atoms from one compound to another)	Alcohol dehydrogenase: Ethyl Alcohol + NAD↔ acetaldehyde + $NADH_2$
2	Transferases	Transfer of functional groups (such as phosphate groups, amino groups, methyl groups)	Hexokinase: D-Hexose + ATP ↔ D-Hexose-6-phosphate
3	Hydrolyses	Hydrolyses reactions (addition of water molecule to broke a chemical bond)	Lipase: Triglyceride + H_2O ↔ diglyceride + a fatty acid
4	Lyases	Addition to double bonds in a molecule as well as non hydrolytic removal of chemical groups	Pyruvate decarboxylase: Pyruvate ↔ acetaldehyde + CO_2

Major classes of Enzymes contd…..

Major Classes of Enzymes

Class No	Class Name	Catalytic Reaction	Example of Enzyme and the Reaction it Catalyzes
5	Isomerases	Isomerization reactions (in which one compound is changed into another having the same number of kinds of atoms but differing in molecular structure	Triphosphate isomerase: D-Glyceraldehyde-3-phosphate ↔ Dihydroxyacetone phosphate
6	Ligases	Formation of bonds with cleavage or breakage of ATP (adenosine tri phosphate)	Acetyl-coenzyme A synthetase ATP+acetate+coenzyem A ↔ AMP+pyrophosphate+acet--ylcoenzyme A

Enzyme Substrate Complex:

E + S ↔ ES → E + P

- Typically one enzyme molecule can catalyze the conversion of 10 to 1000 molecules of substrate to products in one second.
- Catalyzed reactions may be several thousands to billion times faster than same reactions without enzymes.
- If enzymes were absent, the break down of protein in human digestive processes would take more than 50 yrs instead of few hours.

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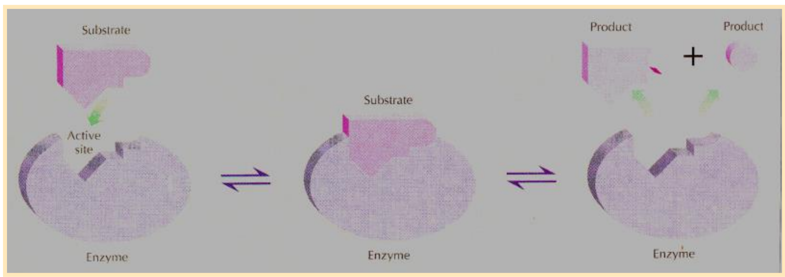
Enzyme-Substrate Reaction

The substrate combines with the enzyme at the active site, which is configured to fit the substrate molecule. In the case illustrated, the chemical groupings of the enzyme-bound substrate are strained so that cleavage of the substrate results . The cleavage products are released form the enzyme, and the enzyme is free to combine with more substrate and repeat the process.



Enzyme Specificity

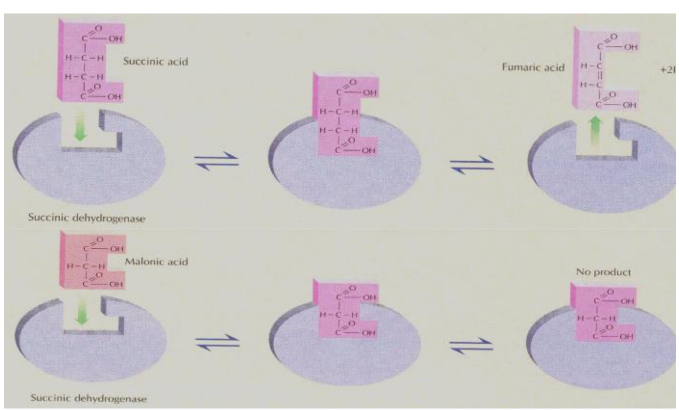
- A single enzyme may react with one substrate or at the most with a group of closely related substrates.
- Each enzyme causes a one step change.
- Most biological processes thus require a form of cooperation among groups of enzymes, rather like a relay team running a long race.
- Enzyme specificty is based to a great extent on the three dimensional structure of the active site on the enzyme molecule.
- Specifically related to L & D isomer (left hand glove to right hand glove)

Enzyme Inhibition
By physical or chemical conditions

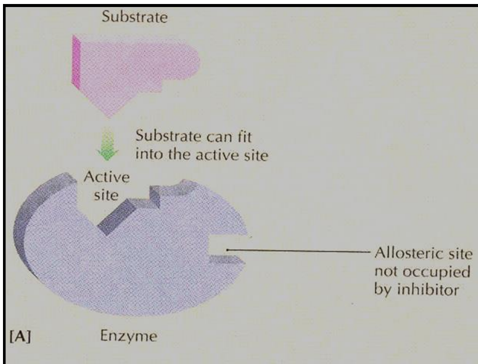
- Competitive inhibition
- Non competitive inhibition
- Feedback inhibition → Allosteric inhibition: a non competitive inhibition in which an inhibitor (in this case the product molecule) binds to the enzyme at some place other than the active site. This distorts the active site so that the substrate no longer fits into it.
- Lowers enzyme activity

Competitive Inhibition

Competitive inhibition (schematic diagram) of the enzyme succinic dehydrogenase by malonic acid. Malonic acid has a structure that is similar to that of the substrate, succinic acid, allowing it to compete with the substrate for attachment to the active site on the enzyme surface. If malonic acid occupies the site, further enzyme activity is blocked, as malonic acid is not changed by this enzyme.

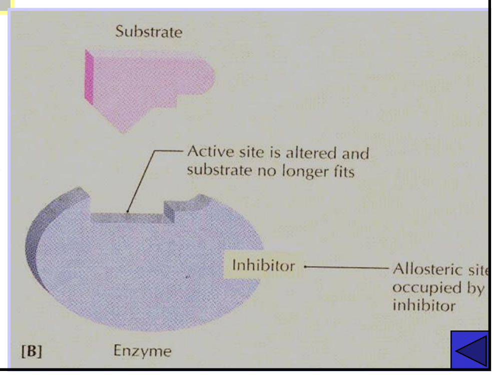


Allosteric inhibition of an enzyme.



[A] An allosteric site is located at a region on the enzyme other than the active site. When the allosteric site is unoccupied, the substrate for the enzyme can fit into the active site.

[B] When a specific inhibitor occupies the allosteric site, the active site is distorted and substrate no longer fits.



Microbiology and Microbial World

- Look in any direction, and you will see signs of microorganisms at work
- Found nearly everywhere, microorganisms are the most widely distributed group of organisms on earth
- You are a home to roughly 100 trillion microorganisms
- MOs are on your skin and hair, in the tartar on your teeth, along your intestine, and elsewhere on body surfaces
- Bacteria are responsible for oxygen built-up in atmosphere, as well as capturing nitrogen from the air
- Bacteria and fungi degrade residues (waste) such as dead plants, discarded food, oil from spills, and human and animal excreta.
- Food production, drug manufacturing, and other industries frequently utilize microorganisms or their by-products

Microbial World and Microbiology

- Every gram of waste material your body discharges from the large intestine contains 10 billion microorganisms, which are quickly replaced by others
- No other organisms have the ability to chemically alter substances in as many ways as do microorganisms
- Chemical changes caused by microorganisms are called biochemical changes, because they involve living organisms
- Some of these biochemical reactions are the same as those in other forms of life, including humans
- Such similarities, coupled with the convenience of studying microbes, make these organisms important in research
- Chemists, physiologists, geneticists, and other frequently use microbes to explore the fundamental process of life

Microbiology

- Concerned with all aspects of microorganisms
  - Structure; nutrition; reproduction; heredity; chemical activities; classification and identification
  - Distribution and activities in nature; relationship to each other and to other organisms, and ability to cause physical and chemical changes in the environment
  - How the microorganisms affect the health and welfare of all life on the earth.

Basic and Applied Microbiology

Cells and Cell Theory

- The word *cell* first appeared in 1665, when an Englishman, Robert Hooke, used it to describe plant materials he saw through his microscope looking at thin slice of cork, he noted the honeycomb like structures formed by the walls of once-living cells.
- On this basis and other observations, two German scientists (*Matthias Schleiden and Theodore Schwann*) developed the *cell theory* in 1938-39.
- They suggested that cells are the basic structural and functional units of all organisms
  - From single celled microorganisms (MO) to life forms with specialized tissues and complex organ system
- As the cell theory gained acceptance, investigators speculated about the substance within the cell, *protoplasm* (the "first formed substance").

Protoplasm

Is a complex, gelatinous mixture of water and organics (carbohydrates, proteins, lipids and nucleic acids), enclosed by flexible membrane and sometime by rigid cell wall.

Nucleus

- Region that controls cell function and inheritance
- Contains coded information
- Surrounded by membrane (in some cells), nuclear membrane
- Nucleoid, no membrane (in some cells)

Cytoplasm

- Non nuclear area remainder of protoplasm

All the life processes take place within the cell in a unicellular organism, while in multicellular organism each cell/tissue/organism performs a specific function

Organisms' Basic Characteristics

- Reproduce
- Use food as source of energy
- Synthesize cell substance and structures
- Excrete wastes
- Respond to change in the environment
- Mutate, through infrequent, sudden changes in their hereditary characteristics.

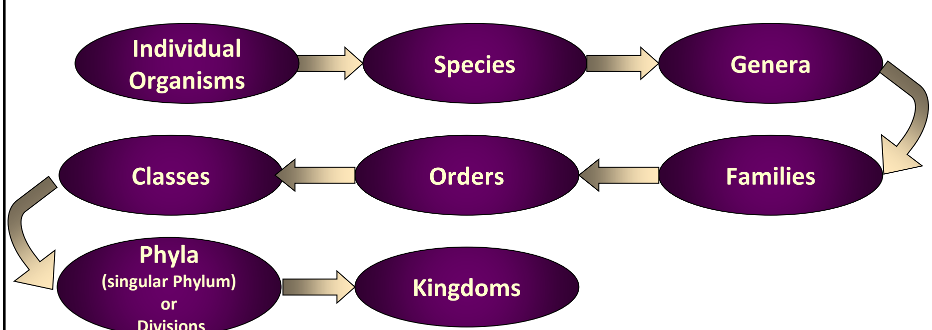
Classification of Living Organisms

- There are about 10 million species of living organisms in the world, including thousands of microbial species
- The need to make order out of this great number and variety of organisms is characteristics of human mind
- Placed into groups based on their similarities
- The science of taxonomy includes classification (arrangement), nomenclature (naming), and identification (description and characterization) of living organisms
- Organisms that share certain common characteristics are placed into taxonomic groups called texa (singular taxon)
- The basic Taxon is the species

Some Examples of the Classification of Organisms

		Organism	
Taxa (categories)	Cat	Alga	Bacterium
Kingdom or Major Group	Animal	Plant	Eubacteria
Division		Chlorophyta	Gracilicutes
Phylum	Chordata		
Subphylum	Vertibrata		
Class	Mammalia	Chlorophyceae	Scotobacteria
Subclass	Eutheria		
Order	Carnivora	Volvocales	Spirochaetales
Family	Felidae	Chlamydomonadaceae	Leptospiraceae
Genus	Felis	Chlamydomonas	Liptospira
Species	*F. domesticus*	*C. eugametos*	*L. interrogans*

Hierarchy in Classification of Organisms



Species

- Collection of strains with similar characteristics in their hereditary material (a strain is made up of the descendants of a single colony from a pure culture)
- Features used to place organisms into species include morphology and nutritional requirements

Genera (singular genus)

Group of closely related species

Classification of Living Organisms

- Name of the species is always given as a two-part Latin combination (binomial) → Consisting of the genus name and a specific name that denotes the species.
  - *Homo sapiens* or *H. sapiens*
  - *Escharichia coli* or *E. coli*
- Because of different traditions among the various biological sciences, there is no consensus on the nomenclature and classification of every taxon.
- Zoologists and botanists, agree with few exceptions, on the general arrangement of animals and plants into phyla/divisions but microbiologists have not established phyla that satisfy bacteriologists, phycologists, protozoologists, and others.
  - Partly because of this lack of agreement, the genus and the species remain the two most important texa among bacteria.

Classification of Living Organisms

- During mid-eighteenth century, Carolus Linneeus developed the binomial system and placed all living organisms in two kingdoms, Plantae or Animalia.
  - Pioneering work, great scientific work, however, classification systems were misleading or just plain wrong because they were based on inaccurate information.
- Classification systems, particularly those for MO's are still evolving as more discoveries are made about physical and chemical characteristics.
- Two kingdom classification for MO's Protozoa in animal & other MO's in Plants.
  - Concept is impractical, particularly for microorganisms, as some have characteristics of both, plant and animal.

Classification of Living Organisms

- In 1866 Ernst H Haeckel (Student of Charles Darwin) proposed a third kingdom called Protista for those MO's who have features of both plants and animals, and included bacteria, algae, yeasts, and protozoa in this.
  - ➢ But validity of this kingdom is questioned as more information about the internal structures of microbes became available.

Procaryotic & Eucaryotic Microorganisms

- Based on how nuclear substance exists within the cell (An important discovery in terms of taxonomy for microbial cells based on advances in electron microscopy).
  - Bacteria are procayrotes (major criteria for separation from other microbes)
  - Algae, fungi, protozoa and cells of plants and animals have eucaryotic cell structure and are eucoryotes.

The Five-Kingdom Concept of Classification

- The Five-Kingdom Concept Based on Obtaining Nutrition from Food (By Whittaker in 1969): Three levels of cellular organization have evolved to accommodate three principle modes of nutrition.
  - Photosynthesis (light energy to convert CO_2 to sugars)
  - Absorption (uptake of chemical nutrients dissolved in water)
  - Ingestion (intake of insoluble particles of food)

The Five-Kingdom Concept of Classification

1. **Monera** (prokaryotes → bacteria): Considered the most primitive kingdom and thought to be the ancestors of the eucaryotes.
2. **Protista** (Eucaryotes → unicellular MO's, principally algae and protozoa, and also the slime molds, the lower fungi) →Represent all three categories of food intake. (Algae → photosynthesis; Protozoa → ingest; Slime molds → only absorb)
3. **Plantae** → higher eucaryotic organisms which are photosynthetic → green plants & higher algae.
4. **Animalia** → Animals which ingest food.
5. **Fungi** → organisms that have cell walls but lack the photosynthetic pigment chlorophyll found in other plants and thus absorb their food.

Archaeobacteria, Eubacteria, and Eucaryotes

- Monera & Protista → considered as ancestral forms; pro → earlier than; → Different ancestral pattern, established now based on rRNA.

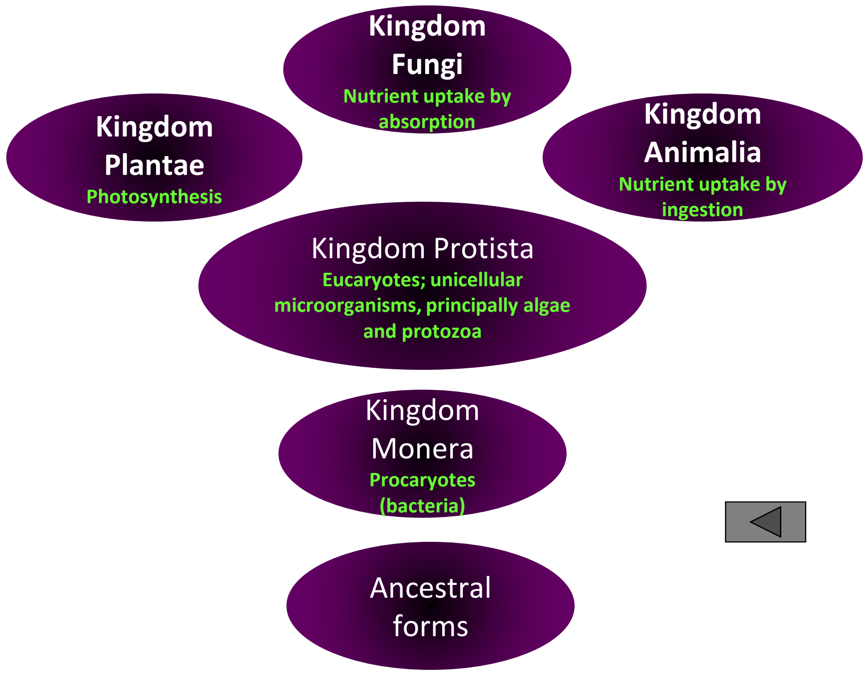
- Archaeobacteria, eubacteria, and eucaryotes evolved through separate pathways from a common ancestor.
- The Endosymbiotic Theory

Major Schemes of Classification of Living Organisms

Classification scheme	Kingdoms	Organisms included
Linnaeus (1753)	Plantae	Bacteria, fungi, algae, plants
	Animalia	Protozoa and higher animals
Haeckel (1865)	Plantae	Multicellular algae and plants
	Animalia	Animals
	Protista	Microorganisms, including bacteria, protozoa, algae, molds, and yeasts
Whittaker (1969)	Plantae	Multicellular algae and plants
	Animalia	Animals
	Protista	Protozoa and single-celled algae
	Fungi	Molds and Yeasts
	Monera	All bacteria (procaryotes)

Major Schemes of Classification of Living Organisms

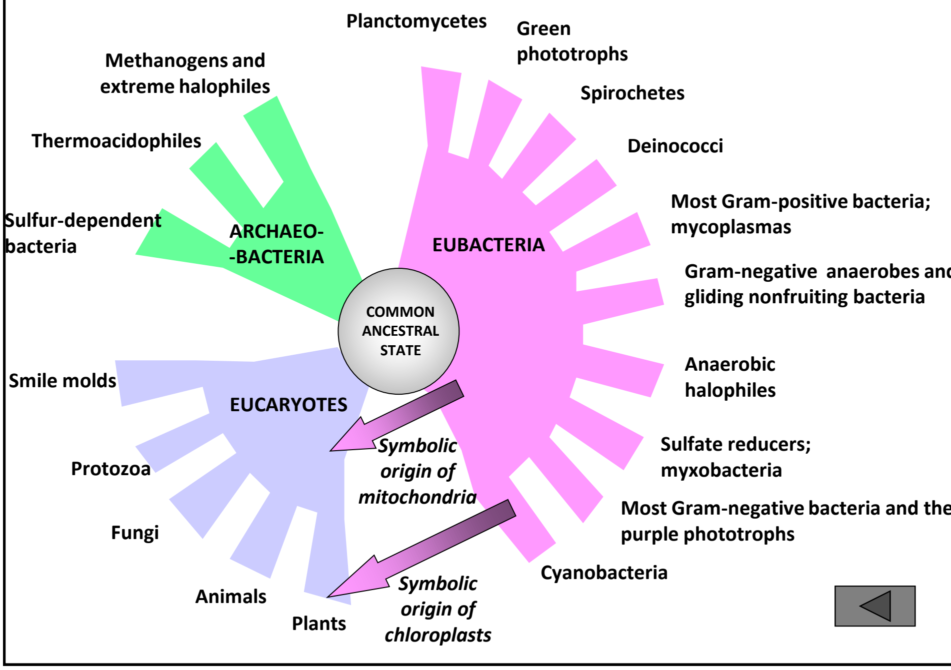
Classification scheme	Kingdoms	Organisms included
Woese (1977)	Archaeobacteria	Bacteria that produce methane gas, require very high levels of salt, or require very high temperatures
	Eubacteria	All other bacteria, including those most familiar to microbiologists, such as disease-causing bacteria, photosynthesic bacteria
	Eucaryotes	Protozoa, algae, fungi, plants, and animals



Simplified scheme of Whittaker's five-kingdom classification of living organisms

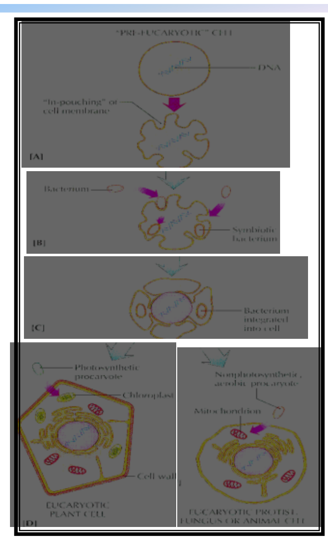
Classification Based on Depiction of the Pathways Through which Living Organisms Evolved

A depiction of the pathways by which living organisms evolved, is deduced from comparative studies of ribosomal RNA. The three major evolutionary branches are shown leading to present-day archaeobacteria, eubacteria, and eucaryotes. Within the eubacterial branch, at least 10 distinct lines of descent occurred; in the archaeobacteria branch, at least three distinct lines of descent occurred. In the case of eucaryotes, there is evidence that certain Gram-negative eubacteria invaded a primitive from of eucaryotic cell and evolved as specialized intracellular organelles called *mitochondria*. Chloroplast, the photosynthetic organilles of plant cells, appear to have evolved in a similar manner from cynobacteria.



The Endosymbiotic Theory

Proposes the manner in which eucaryotic cells may have evolved. This theory suggests that a “pre-eucaryotic” cell developed an “in-pouching” of the cell membrane [A]. Bacteria entered the “in-pouched” area as symbionts [B] and became an integral part of the cell [C]. When the bacterial symbiont was a photosynthetic procaryote, it functioned as a chloroplast and a plant cell evolved. When the bacterial symbiont was a nonphotosynthetic aerobe, it functioned as a mitochondrion (providing energy) and an animal or protist type of cell evilved [D].



Some Differential Characteristics of Procaryotes and Eucaryotes

Characteristic	Procaryotes	Eucaryotes
Genetic material seperated from cytoplasm by a membrane system	No	Yes
Usual cell width or diameter	0.2 to 2.0 µm	>2.0 µm
Mitochondria	Absent	Present
Chloroplast (in photosynthetic species)	Absent	Present
Endoplasmic reticulum and Golgi complex	Absent	Present
Gas vacuoles	Formed by some species	Absent
Poly-*β*-hydroxylbutyrate inclusions	Formed by some species	Absent
Cytoplasmic streaming	Absent	Often Present
Ability to ingest insoluble food particles	Absent	Present in some species
Flagella, if present:		
▪ Diameter	0.01 to 0.02 µm	Ca. 0.2 µm
▪ Cross section shows "9+2" arrangement of microtubules	No	Yes

Some Differential Characteristics of Procaryotes and Eucaryotes Contd….

Characteristic	Procaryotes	Eucaryotes
Heat resistant spores (endospores)	Formed by some species	Absent
Polyunsaturated fatty acids or sterols in membranes	Rare	Common
Muramic acid in cell well	Common	Absent
Ability to use inorganic compounds as a sole energy source	Present in some species	Absent
Ability to fix atmospheric nitrogen	Present in some species	Absent
Ability to dissimilate nitrates to nitrogen gas	Present in some species	Absent
Ability to produce methane gas	Present in some species	Absent
Site of photosynthesis, if it occurs	Cytoplasmic membrane extensions; thylakoids	Garna of chloroplasts
Cell division occurs by mitosis	No	Yes
Mechanisms of Gene transfer and recombination, if they occur, involve gametogenesis and zygote formation	No	Yes

Some Differential Characteristics of Procaryotes and Eucaryotes Contd….

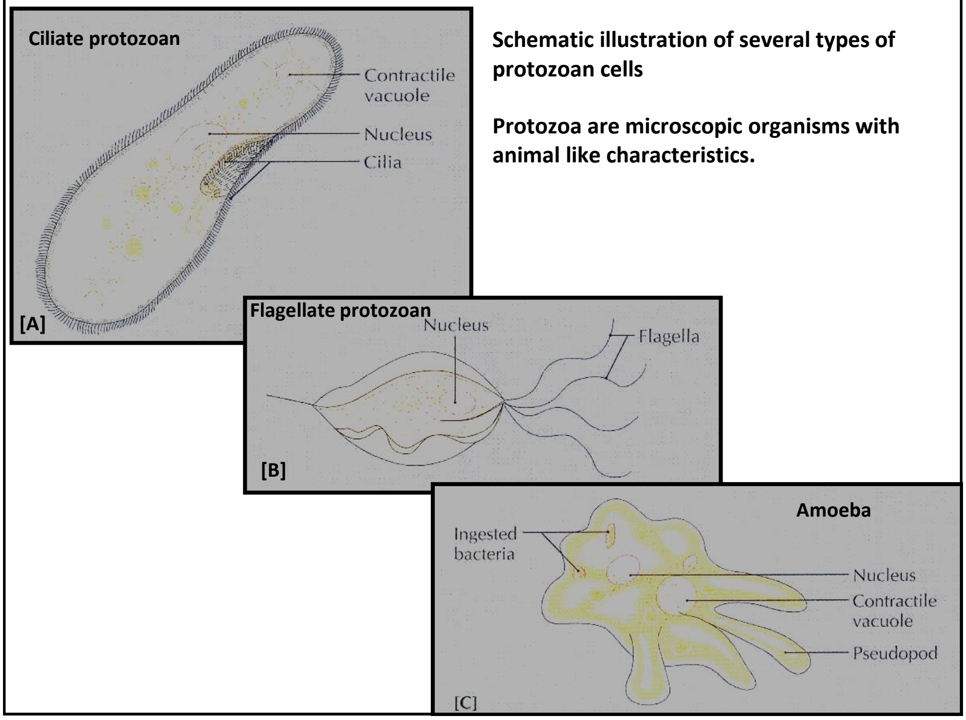
Characteristic	Procaryotes	Eucaryotes
Chromosomes:		
▪ Shape	Circular	Linear
▪ Number per cell	Usually 1	Usually > 1
Ribosomes:		
Location in cell	Dispersed throughout cytoplasm	Attached to endoplasmic recticulum
Sedimentation constant (in Svedberg units)	70 S	80 S*

* Except in mitochondria and chloroplast, which have ribosomes of the procaryotic type (70 S)

The Major Groups of Organisms Based on Certain Traits (Characteristics or Actions)

Protozoa, Fungi, Algae, Bacteria; and Viruses.?

- **Protozoa:**
  - Single celled, Eucaryotic MOs
  - Animal like (ingest food), lack of rigid cell wall. Do not contain chlorophyll.
  - Some can swim through the beating action of short hair like appendages called flagella.
  - Other protozoa, do not swim but can creep along surfaces (amoebas).
  - Other type, called sporozoans **>** they form resting bodies, called spores during one phase of their life cycle **>** usually non-mobile in this phase
  - Occur widely (in nature)
  - Some cause animal and human diseases
  - Some are beneficial, help digesting the food in some animals.



Schematic illustration of several types of protozoan cells

Protozoa are microscopic organisms with animal like characteristics.

- **Algae**:
  - Considered plant like, contain green pigment chlorophyll, carry out photosynthesis, have rigid cell walls.
  - These are eucaryotes, may be unicellular and microscopic or multicellular and upto several meters in length.
  - Problems: clogging, releasing toxics, growing in swimming pools, etc.
  - Uses (extracts from some algae): Thickeners and emulsifiers for foods such as ice creams, custards, as an anti inflammatory drugs for ulcer, as a source of agar.

Fungi

- Eucaryotic like algae (single or multicellular) (mushrooms and bracket fungi growing on damp layers of soil)
- Do not contain chlorophyll --- can not carry out photosynthesis.
- Absorb dissolved nutrients.
- Fungi (which are MOs) with multicells, producing filamentous microscopic structures are called molds > Cells are cylindrical in shape and are attached end to end, thread like called hyphae >Individually hyphae are microscopic in size > When large number accumulate (say on slice, food, etc.), the moldy mass called mycelium, is visible to naked eye. > Molds have considerable value: used to produce antibiotic penicillin; soy sauce, cheeses and many other products, biosorption > Responsible for deterioration of materials such as textiles, woods, etc., Cause diseases in animals, plants and humans, (Peanuts spoilage).
- Yeasts are unicellular fungi > Shapes: Spherical to ovoid; ellipsoidal to filamentous > Both beneficial and detrimental: Used in baking industry (produce gas and makes dough rise), alcohol production, spoil food and cause diseases.

Bacteria

- Procryotes > Eubacteria and archaeobacteria (Based on ribosomal RNA differences)
- Eubacteria: Unicellular; Variety of shapes; 0.5 to 5 μm; often appear in pairs, chains, tetrads (group of four) or clusters; Essential in recycling wastes and production of antibiotics such as streptomycin; Cause diseases > sore throat, tetanus, plague, cholera, tuberculosis.
- Archaeobacteria: Have ability to survive in unusually harsh environments (high temperature, acids, salts, etc.); Some are capable of unique chemical activity > e.g. production of CH_4 from CO_2 & H_2 (live in environments with no oxygen, such as deep in swamp mud, or in the intestines of ruminants such as cattle and sheep).

Viruses

- Border line between living and non living; not cells (much smaller 20 to 300 nm); simple in structure; get into the genetic material and damage the cell; Cause variety of diseases such as AIDS (HIV), common cold; genital herpes; poliomyelitis; hepatitis, tobacco mosaic (disease of tobacco plant), etc.; Also implicated for growth of some malignant tumors; Contain only one type of nucleic acid RNA or DNA, surrounded by protein coat.

Microorganisms and the Environment

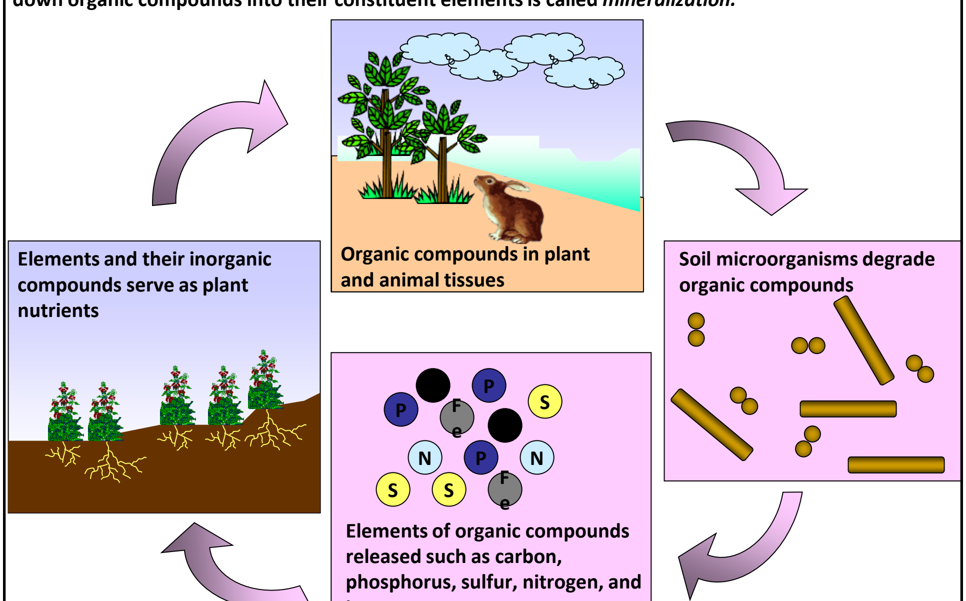
- Microorganisms are everywhere
  - Air current carries them to upper atmosphere and from continent to continent
  - Microorganisms inhabit all marine environments, from surface waters to the bottom of ocean trenches
  - There may be billions of them in a tea spoon of fertile soil
  - Total mass of microbial cell on the earth = 25 times the total mass of animal life
  - Animals carry large population of microbes on their body surfaces, in the intestinal track, and in their body openings.

Microorganisms and the Environment

- Human body contains 10 trillion cells and 100 trillion microorganisms
  - 10 microorganisms for each cell
  - Bacteria aid in digestion and account for 50% of weight of human and animal feces
  - Relatively few can cause disease; however, they have created an impression that all microorganisms are germs and are harmful.
- Microorganisms play a key role in recycling of elements in nature.

*The food chain, animal eats plants and other animals, and plant use animal wastes for nutrients; but microorganisms act as **translators** in this process.*

A schematic illustration of the role of microorganisms in the recycling of compounds and elements (natural resources) in nature. Elements bound in complex organic molecules are released by the metabolic activities of microorganisms and made available as plant nutrients. The process of breaking down organic compounds into their constituent elements is called *mineralization.*



Microbiology as a Science

- There are two major areas of study in the field of microbiology: basic microbiology, where the fundamental nature and properties of microorganisms are studied, and applied microbiology, where information learned from basic microbiology is employed to control and use microorganisms in beneficial ways.

Basic Microbiology

- Morphological characteristics: Shape, size, chemical composition and functions of internal structures.
- Physiological characteristics: Specific nutritional requirements and physical conditions needed for growth and reproduction.
- Biochemical activities: Degradation and synthesis reaction.
- Genetic characteristics: Inheritance and the variability of characteristics.
- Disease causing potential: Presence/absence for human, animals, plants, includes the study of host resistance to infection.
- Ecological characteristics: Natural occurrence and Interaction with others and environment.
- Classification: Taxonomic studies.

Applied Microbiology

Useful applications of microbiology are unlimited in their scope and variety

Medicine,
food (Single cell proteins, SCPs)
dairy products,
agriculture,
industry or the environment

Characterization of Microorganisms

- Under natural conditions microbial populations contain many different species
  - Not only of bacteria, but also of yeasts, molds, algae, protozoa, etc.
- Frequently it is important to identify how many and what kinds of MOs are present in a particular environment.
- We must have techniques to isolate, enumerate, and identify the microbes in a sample of material.

Pure Culture Techniques

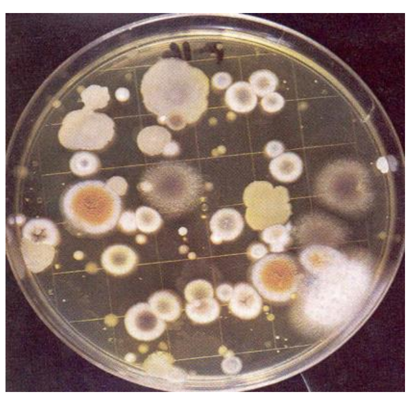
- Isolation and cultivation of pure cultures.
- Preservation of pure cultures.
- 4 to 10 ^{o}C storage.
- May have to be transferred every day in new media.
- For long term storage, cultures are kept in tanks of liquid nitrogen (–196 ^{o}C), or in the freezers at –70 to –120 ^{o}C, or frozen and then dehydrated and sealed under vacuum in a process called lyophilization (freeze drying).

Identification of MOs

- Involve different techniques that may include the use of different media and different chemical reactions, but one of the powerful detective techniques is microscopic examination.

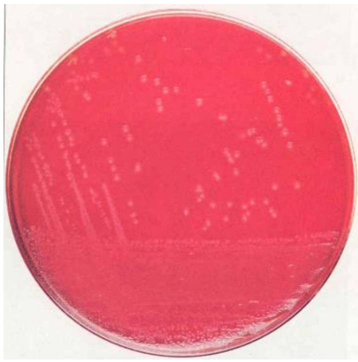
Microscopic Examination

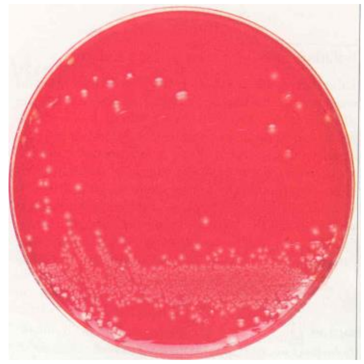
- **Light or Optical Microscope**
  - 1000 or at the most 2000 times (resolving power; 0.25µm)
  - Bright field
    - Uses a direct light source that illuminates the entire specimen field
    - Since the microorganisms are transparent, they do not stand out distinctly with this type of microscopy
    - Microbiologists usually stain, or color with dye, those microorganisms viewed with bright field microscopy.
  - Dark field
    - Uses a light microscope, but brightly illuminates the microorganism against a dark background
    - Looks like a dancer in a spotlight on a stage against a black curtain
  - Fluorescence microscopy
    - Specimen is stained with a fluorescent dye
    - Fluorescent antibody test.
  - Phase contrast microscopy
    - Light microscopy that permits greater contrast



Colonies of microorganisms that have grown on a nutrient agar plate after being exposed to room air

Plate culture techniques for isolation of microorganisms in pure culture

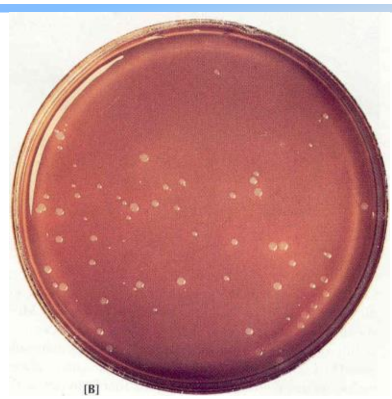




Streak-plate Method

The specimen is streaked onto the surface of the agar medium with a loop needle to thin out the population so that on some regions cells will later grow into isolated colonies

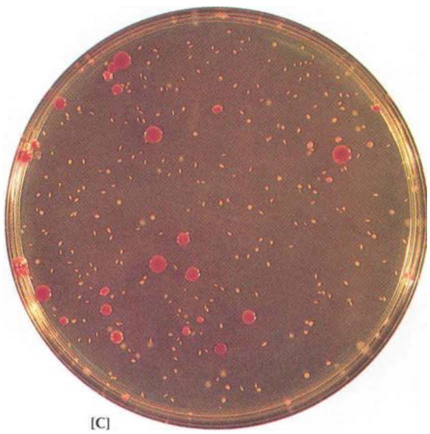
Plate culture techniques for isolation of microorganisms in pure culture



Spread-plate Method

A drop of diluted sample of the specimen is placed on the surface of an agar medium, and this drop is spread over the entire surface using a sterile bent glass rod.

Plate culture techniques for isolation of microorganisms in pure culture



Pour-plate method

The specimen, in this instance a culture of *Serratia marcescens,* is diluted by addition to tubes of melted (cooled) agar media. After through mixing, the tubes of inoculated media are poured into sterile Petri dishes; after solidification they are incubated. In this procedure colonies will grow both on and below the surface (subsurface colonies), since some cells are trapped within the agar medium when it solidifies.

In each of these techniques the objective is to thin out the microbial population so that individual cells are located at a distance from other cells. The individual cells, if far enough apart, will produce a colony that does not touch other colonies . All the cells in a single colony have the same parentage. To isolate a pure culture, a transfer is made from an individual colony onto a medium in a test tube.

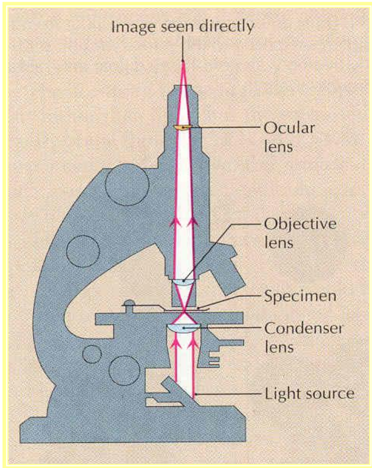
Identification of MOs

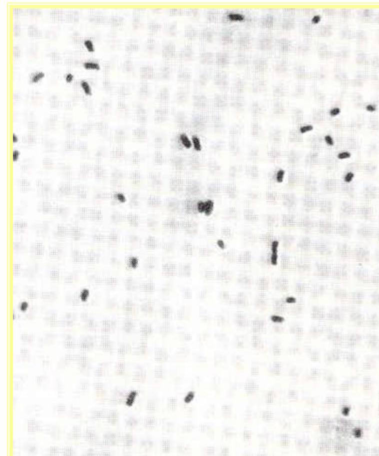
- Electron Microscope
  - Permits greater magnifications because of greater resolving power.
  - Beam wavelength in the range of 0.005 to 0.003 nm.
  - Resolving power 0.003µm as against 0.25µm in light microscopy
  - Scanning electron microscopy (SEM)
  - Transmission electron microscopy (TEM)

A microscope's useful magnification is limited by its resolving power, or its ability to distinguish images of two close objects as separate, distinct entities. The resolving power is a function of the wavelength of light and the numerical aperture of the lens system. Light microscopes, by using visible light have a resolving power of approximately 0.25 µm

- Preparing Microorganisms for Light Microscopy
  - Wet-mount and Hanging-drop techniques
  - Staining technique: Simple staining, differential staining, gram staining.

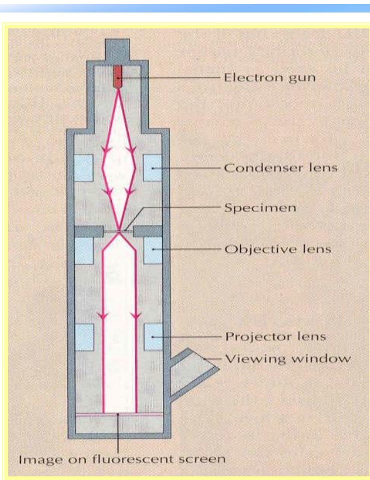
Diagrammatic Comparison of Imaging Systems
Optical Microscope

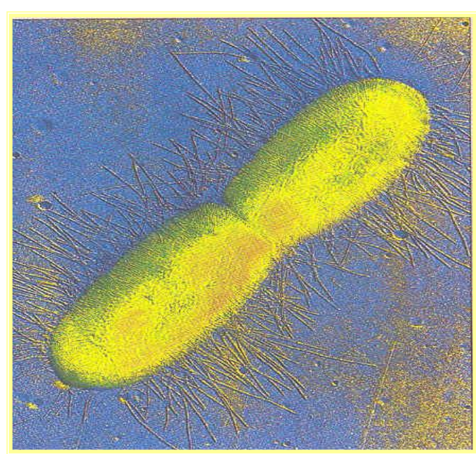




10 µm

Diagrammatic Comparison of Imaging Systems
Transmission Electron Microscope (TEM)



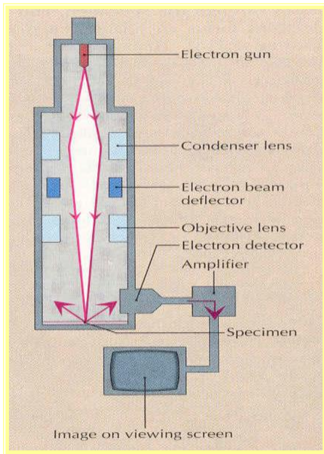


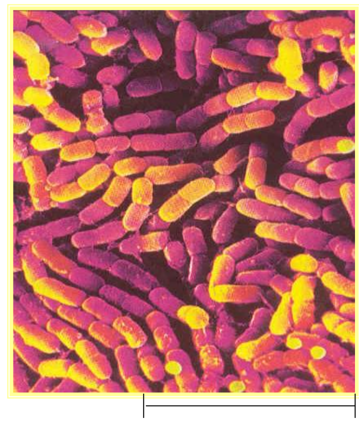
0.1 µm

Diagrammatic Comparison of Imaging Systems

Scanning Electron Microscope (SEM)

The appearance of the bacterium *Escherichia coli* is shown in each type of microscope





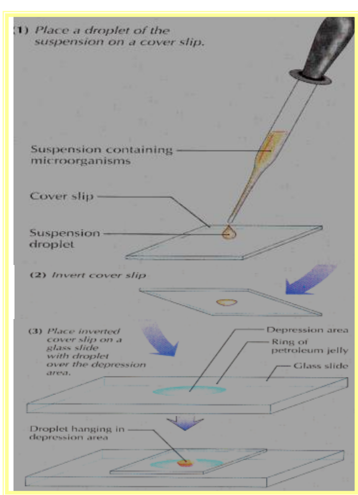
10 µm

A Comparison of Different Types of Microscopy

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful applications
Bright-field	1000-2000	Specimens stained or unstained; bacteria generally stained and appear color of stain	Gross morphological features of bacteria, yeasts, molds, algae, and protozoa
Dark-field	1000-2000	Generally unstained; appears bright or "lighted" in an otherwise dark field	Microorganisms that exhibit some characteristics morphological feature in the living state and in fluid suspension, e.g., spirochetes
Fluorescence	1000-2000	Bright and colored; color of the fluorescent dye	Diagnostic techniques where fluorescent dye fixed to organism reveals the organism's identity

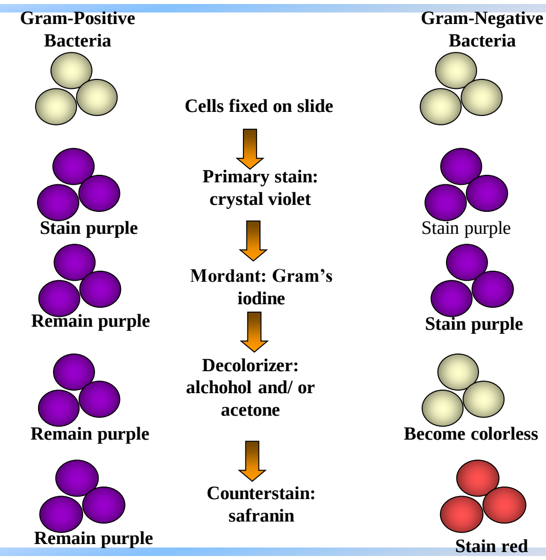
A Comparison of Different Types of Microscopy

Type of microscopy	Maximum useful magnification	Appearance of specimen	Useful applications
Phase-contrast	1000-2000	Varying degrees of “darkness”	Examination of cellular structures in living cells of the larger microorganisms, e.g., yeasts, algae, protozoa, and some bacteria
Electron	200,000-400,000	Viewed on fluorescent screen	Examination of viruses and the ultra-structure of microbial cells



Schematic illustration of the preparation of a hanging drop slide

The color appearance of bacterial cells at each step of the gram stain procedure.

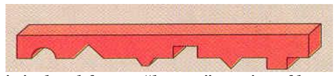


Information Used to Characterize Microorganisms

Microscopic to analysis of genetic material.

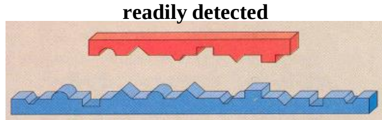
- Morphological characteristics: Shape, size, arrangement, and structure of both whole cells and internal components
- Nutritional and cultural characteristics
- Metabolic characteristics
- Antigenic characteristics
  - Antigen
  - Stimulates the production of antibodies
- Pathogenic characteristics
- Genetic characteristics

Schematic illustration of the principle of the DNA probe technique of the identification of bacteria

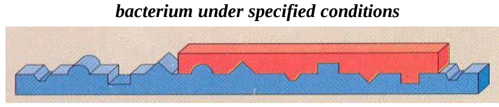


A piece (strand) of DNA is isolated from a "known" species of bacteria. If the nucleotide sequence of this strand of DNA is unique to the species, the strand can be used as a DNA probe

The DNA strand is labeled with a radioactive element or other substance that can be readily detected



The probe is mixed with a DNA strand that has been isolated from an unknown bacterium under specified conditions



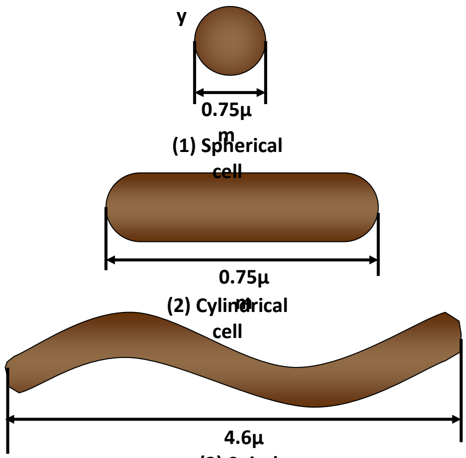
If the DNA probe and strand combine to form a duplex (double stranded DNA), the unknown bacterium is one of the same species as that from which the probe was isolated

Procaryotic Cell Structure

- **Gross Morphological Characteristics**
  - Size
    - 0.5 to 2 µm in diameter/width & 2-3 to 100 µm length
      - High Surface Area to Volume Ratio
  - Shape
    - Three basic shapes: spherical (coccus/cocci);
    - cylindrical or rod like (*bacillus/bacilli*);
    - spiral or helical (*spirillum/spirilla*)

Relative Sizes of three types of bacteria shown:

Schematically



Microscopically

10 µm

Bacteria are generally either:

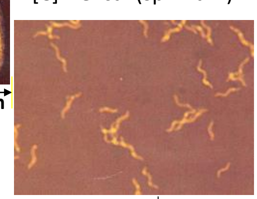
[A] Spherical (coccus),

5 µm

[B] cylindrical (bacillus)

0.1 µm

[C] Helical (spirillum)



5 µm

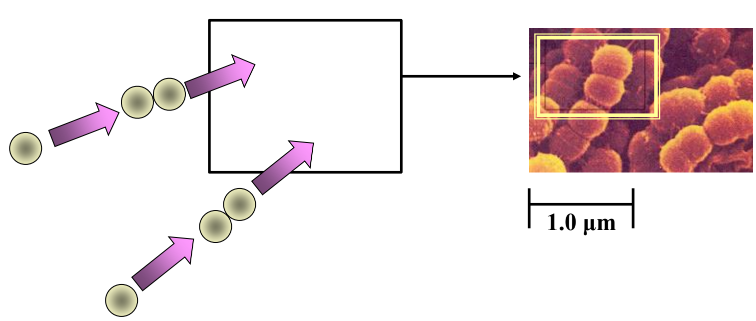
Bacteria are generally either [A] spherical (coccus), [B] cylindrical (bacillus) or [C] helical (spirillum). However, there are many modifications of these three basic forms.
Micrographs show;
[A] *Staphylococcus aureus*
[B] *Klebsiella pneumoniae*
[C] *Aquaspirillum itersonii (negative stain)*

Procaryotic Cell Structure

- Arrangements
  - Often attached to each other;
  - Grow in characteristic arrangement or patterns;
  - Cocci can grow in several arrangements depending on the plane of cellular division and daughter cells staying together following cell division;
    - Is typical of a particular species and can be used for identification;
  - *Diplococcus* (OO) and *Streptococcus* (in chain: OOOOOO);
    - divides in one plane and remains attached after several cell divisions;
  - Tetrads: *tetracoccus*, divide in two planes (groups of four);
  - Division in third plane can result in cubical packets of eight cells known as *sarcinae*;
  - Division in three planes in an irregular arrangement (grapelike cluster)
    - *Staphylococcus*;
  - Bacilli do not generally arrange themselves except a few; match sticks side by side (*diphtheria bacillus*), some in chains (*streptobacilli – Beggiatoa*).

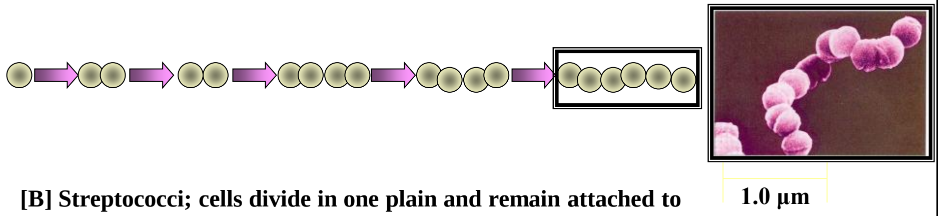
Together, the size, shape, and arrangement of bacteria constitute their gross morphology

Characteristics arrangements of cocci, with schematic illustrations of patterns of multiplication.

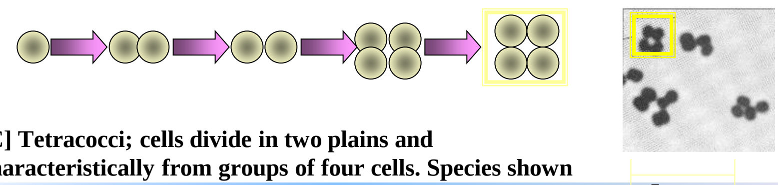


[A] Diplococci; cells divide in one plain and remain attached predominantly in pairs (scanning electron micrograph)

Characteristics arrangements of cocci, with schematic illustrations of patterns of multiplication.



[B] Streptococci; cells divide in one plain and remain attached to form chains (scanning electron micrograph)



5 µm

[C] Tetracocci; cells divide in two plains and characteristically from groups of four cells. Species shown is *Gaffkya tetragena.*

…..How Organisms Grow and Survive

All organisms need the following to survive:

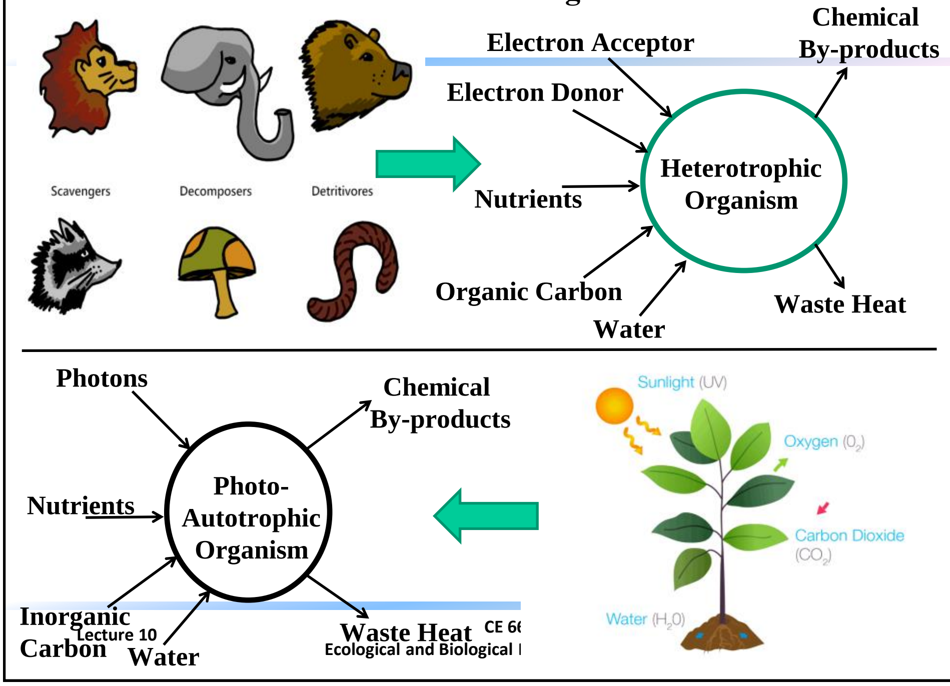
1) Water
2) Carbon
3) Macro Nutrients (N and P)
4) Micronutrients (many other elements)
5) Energy: Obtained by oxidation-reduction reaction involving chemical compounds
   - This process is known as **Respiration**
   - Compound which is oxidized is known as an **Electron Donor**
   - Compound which is reduced is known as an **Electron Acceptor**
6) Electron Donor
7) Electron Acceptor

Source of Carbon

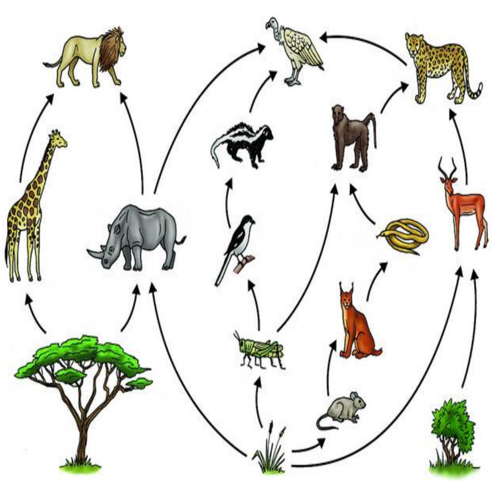
Some organisms have to get organic carbon directly from an external source. These organisms are known as **Heterotrophic**, e.g. Animals.

Some organisms can survive on inorganic carbon obtained from an external source, which they can convert to organic carbon through **Photosynthesis** or other means. These organisms are known as **Autotrophic**, e.g. Plants.

...How Organisms Grow and Survive



The Food Web

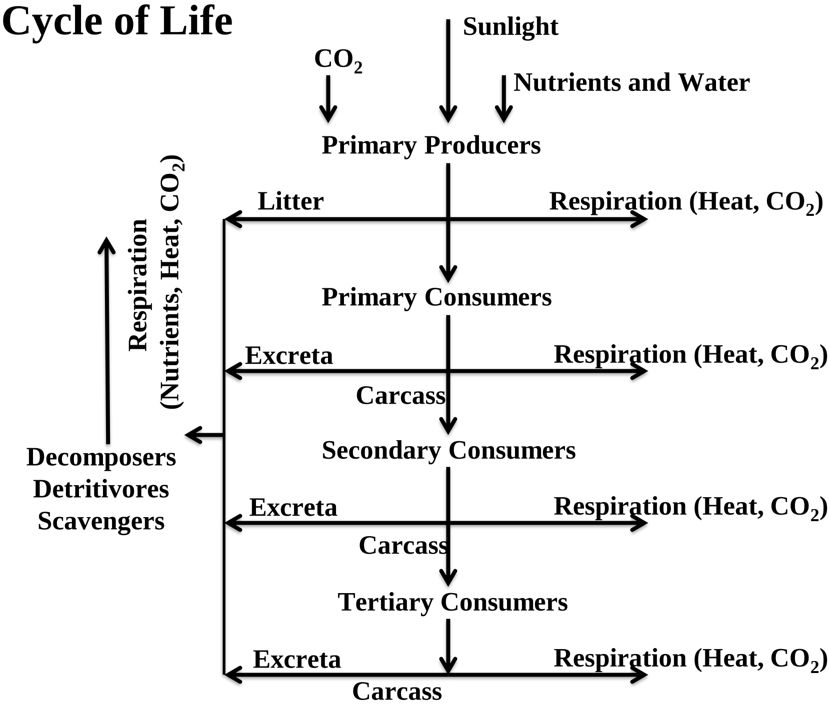


Consumers are organisms of an ecological food chain which receive energy by consuming other organisms. These organisms are formally referred to as heterotrophs which include animals, bacteria and fungi. Such organisms may consume by various means, including predation, parasitization, and biodegradation.

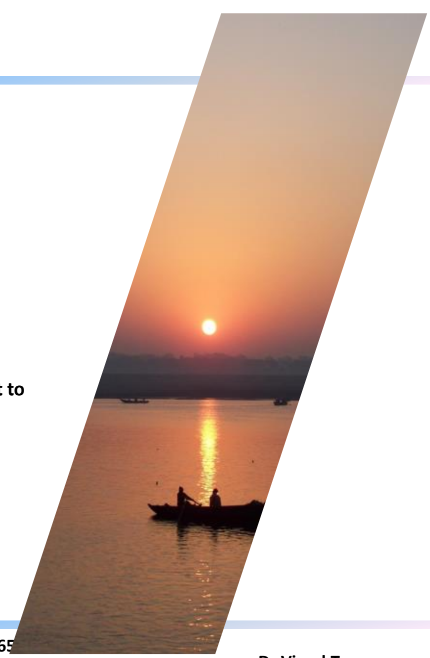
Primary producers are organisms in an ecosystem that produce biomass from inorganic compounds (i.e., autotrophs). In almost all cases these are photosynthetically active organisms (plants, cyanobacteria and a number of other unicellular organisms);

Scavengers/Detrtivors/Decomposers respire on dead and decaying organisms and release CO_2 and nutrients back to the atmosphere.

The Cycle of Life



End of Part 3: Thank You



Questions, Comments & Suggestions may be sent to
vinod@iitk.ac.in

Dr Vinod Tare
Professor & Founding Head-cGanga
Centre for Ganga River Basin Management and Studies
Department of Civil Engineering
IIT Kanpur, UP, India